# Temperature-Driven Dynamics: Exploring Ciliate Feeding Patterns and Microbial Ecosystem Implications

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#### Abstract

Ciliates, such as Tetrahymena Thermophila, are integral components of microbial food webs in freshwater and marine ecosystems, influencing the dynamics of organic matter cycling. This study investigates the temperature-driven dynamics of ciliate feeding patterns and microbial ecosystem implications, focusing on Tetrahymena Thermophila. The study explores the implications of temperature for environmental processes, climate dynamics, and microbial food webs. The methods used in the study include cell counting, phagocytosis analysis, and swimming speed measurement. The results show that there is a strong correlation between temperature and vacuole formation in T. Thermophila. The number of vacuoles increased with temperature, indicating a faster vacuole formation rate. The average speed of the ciliates also increased with temperature. The observed increase in feeding activity at higher temperatures may lead to more efficient cycling of organic matter through microbial communities, with cascading effects on nutrient availability and trophic interactions within aquatic ecosystems. The findings suggest that shifts in temperature regimes, whether seasonal or due to long-term climate trends, could reshape the structure and function of microbial food webs in aquatic environments. Further research is warranted to elucidate the cascading effects of temperature-induced changes in ciliate behavior on broader ecosystem dynamics.

**Keywords:** *Tetrahymena thermophila*, Temperature-dependent feeding patterns, Ciliate behavior, Microbial ecosystems, Microbial dynamics, Climate change, Vacuole formation, Microbial food webs.

# 1 Introduction

Ciliates, such as  $Tetrahymena\ Thermophila^1$ , play a vital role in microbial food webs within freshwater and marine ecosystems. Their rapid movement and consumption of smaller microbes contribute to the cycling of organic matter through microbial food webs. In this experiment, we investigate how temperature impacts the feeding patterns of  $T.\ Thermophila$ , exploring the implications for environmental processes, climate dynamics, and microbial food webs<sup>2</sup>.

## 2 Methods

#### 2.1 Cell count

T. Thermophila cultures were grown in YPD medium + Fe at three temperature conditions: 5, 20, and 30°C. Cell counts were performed using a Bürker counting chamber, with a depth of 0.1 mm. At least 10 squares per sample were counted, and the mean count was used to calculate cell density (cells mL<sup>-1</sup>) using the formula: cells mL<sup>-1</sup> = (Mean count  $\times$  Dilution) / 2.5  $\times$  10<sup>-7</sup>.

## 2.2 Phagocytosis

To evaluate phagocytosis, *T. Thermophila* cultures were mixed with 1% charcoal ink microparticles. Samples were taken at 5, 10, 20, and 30 minutes, and images were captured. Using Fiji from ImageJ,

<sup>&</sup>lt;sup>1</sup>See Plum et al., "Experimental Evolution in Tetrahymena" [1].

<sup>&</sup>lt;sup>2</sup>See Pham et al., "The Effect of Temperature on Food Vacuole Formation in Tetrahymena thermophila" [2], and Luan et al., "The effect of temperature on food vacuole formation by Tetrahymena thermophila" [3].

the number of ink-filled vacuoles was determined. We also used Fiji to measure vacuole size, and the data were analyzed to observe changes over time.

#### 2.3 Swimming speed

Videos of T. Thermophila swimming at different temperatures were recorded and analyzed using Fiji. The "Trackmate" plugin was employed to track cell movement, and the "TRACK\_MEAN\_SPEED" values were converted to  $\mu$ m/s for each condition.

# 3 Results

#### 3.1 Cell Count

We are ashamed to confess that we only managed to recover insufficient data from the different groups regarding the counts and dilution. We were either missing the temperature, the dilution or the count itself. While this is a bit of a setback, we believe that the phagocytosis data and swimming speed data is more insightful and exciting, and hope that it will make up for it!

#### 3.2 Phagocytosis

Displayed below are three pairs of boxplots illustrating the evolution of vacuole number (on the left) and vacuole size (on the right) over a 30-minute interval. These three pairs of graphs represent observations under three distinct temperature conditions: 5°C, 20°C, and 30°C.

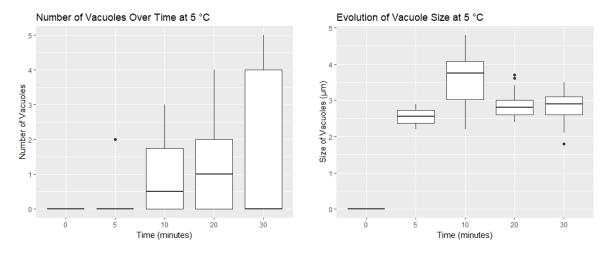


Figure 1: Evolution of vacuole number and size at 5°C. For this particular temperature, a boxplot might not be the clearest plot type since there is a significant proportion of ciliates that do not even have a single vacuole filled with charcoal.

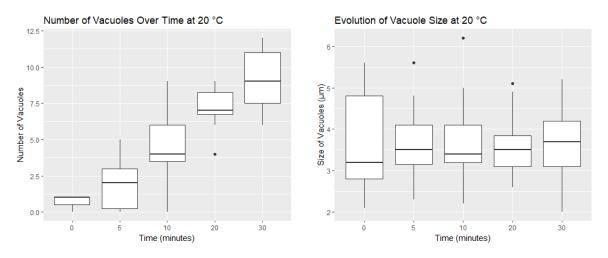


Figure 2: Evolution of vacuole number and size at  $20^{\circ}\mathrm{C}$ 

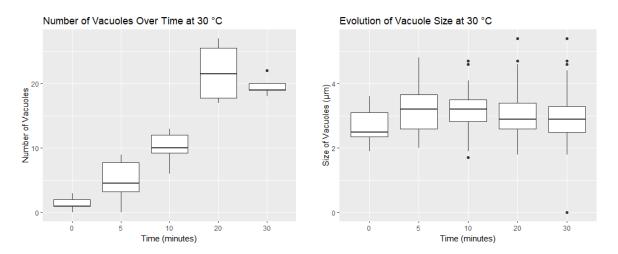


Figure 3: Evolution of vacuole number and size at  $30^{\circ}\mathrm{C}$ 

To summarize our findings regarding vacuole number and size, please find below two summary plots, superposing the boxplots for the 3 temperature conditions:

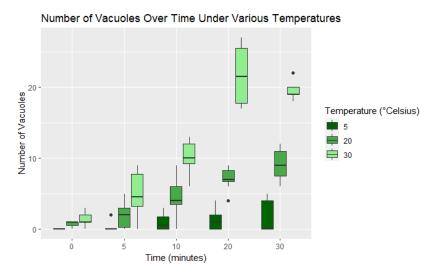


Figure 4: Cumulative plot of the evolution of vacuole number over 30 minutes, under various temperatures

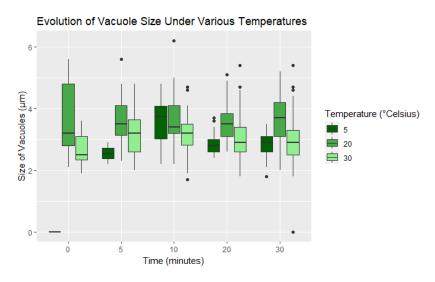


Figure 5: Cumulative plot of the evolution of vacuole size over 30 minutes, under various temperatures

As you can see on the summarized plots above, there is a strong correlation between temperature and vacuole formation in *T. Thermophila*. First, just looking at the number of vacuoles observed, we can notice a major trend: we observe that the number of vacuoles increases with the temperature, which indicates that the speed of vacuole formation increases with temperature. Furthermore, there seems to be an optimal number of vacuoles a little below 20, and an optimal size between 3 and 4 microns, and both are reached faster with higher temperatures. This implies that under warmer conditions, *T. thermophila* not only absorbs charcoal particles more rapidly but also organizes its vacuoles more efficiently for storage.

#### 3.3 Movement

Below is a box plot of the average speed of the ciliates in the videos recorded at the three different temperatures:

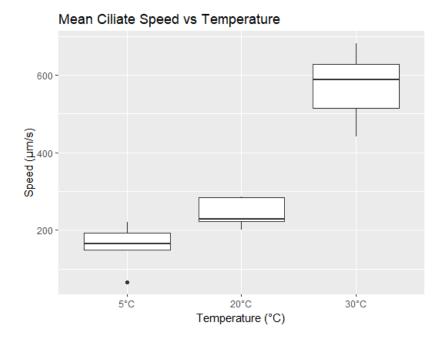


Figure 6: Evolution of mean ciliate speed with temperature. Data was obtained through motion tracking software, as described in the Methods section, and blatant outliers were removed (mainly 0 values due to false positives).

As you can notice, there is a clear positive correlation between the ciliates' speed and the increase in temperature.

#### 4 Discussion

#### 4.1 Temperature Influence on Feeding Patterns

The observed increase in vacuole formation over time at higher temperatures aligns with the known physiological responses of T.  $thermophila^3$ . As a thermophilic ciliate, T. thermophila is adapted to thrive in warmer environments. The faster vacuole formation could be attributed to an accelerated metabolic rate at elevated temperatures, leading to increased phagocytosis and digestion rates. This temperature-dependent response is consistent with the general principle that higher temperatures often enhance metabolic processes in many organisms.

The positive correlation between temperature and feeding patterns has implications for the energy dynamics within microbial food webs. As T. thermophila plays a crucial role in the microbial loop, the increased feeding activity at higher temperatures may lead to more efficient cycling of organic matter through microbial communities. This enhanced microbial processing could have cascading effects on nutrient availability and trophic interactions within aquatic ecosystems.

#### 4.2 Implications for the Environment and Climate

Understanding the temperature-dependent feeding patterns of *T. thermophila* has broader implications for environmental and climate dynamics. With global temperatures on the rise due to climate change, the microbial communities in aquatic ecosystems may experience shifts in the abundance and activity

<sup>&</sup>lt;sup>3</sup>See Weber et al., "Phenotypic responses to temperature in the ciliate Tetrahymena thermophila" [4].

of ciliates like *T. thermophila*. The observed temperature-driven increase in feeding rates suggests that climate-induced changes could influence the efficiency of nutrient cycling in aquatic environments.

Moreover, the implications extend beyond *T. thermophila* to the broader microbial food webs. Changes in the feeding patterns of ciliates can impact the abundance and composition of microbial communities, influencing the balance between autotrophic and heterotrophic organisms. This, in turn, may have consequences for higher trophic levels, including fish and other aquatic organisms that rely on microbial communities as a food source.

## 4.3 Temperature Impact on Ciliate Swimming Speed:

The observed increase in swimming speed at higher temperatures further supports the notion that temperature influences the behavior and physiology of *T. thermophila*. The faster swimming speed could be linked to enhanced motility and prey detection strategies, allowing the ciliates to more efficiently locate and capture food particles.

#### 4.4 Linking Feeding Patterns to Microbial Food Webs

The temperature-dependent changes in feeding patterns and swimming speed of *T. thermophila* underscore the intricate interplay between environmental temperature and microbial dynamics. The microbial food web is a complex network of interactions, and alterations in the feeding behavior of key ciliate species can reverberate throughout the entire ecosystem.

As temperatures rise, microbial processes may become more dynamic, with potential consequences for nutrient cycling, carbon fluxes, and overall ecosystem stability. The findings suggest that shifts in temperature regimes, whether seasonal or due to long-term climate trends, could reshape the structure and function of microbial food webs in aquatic environments.

# 5 Conclusion

All in all, this experiment gives us solid hints regarding the temperature-dependent feeding patterns of T. thermophila and their potential consequences for microbial food webs. The observed increase in vacuole formation and swimming speed at higher temperatures highlights the responsiveness of these ciliates to environmental cues. These findings contribute to our understanding of the intricate links between temperature, microbial dynamics, and ecosystem processes in aquatic environments. Our results only emphasize the need for further research, particularly under realistic environmental conditions, to fully elucidate the cascading effects of temperature-induced changes in ciliate behavior on broader ecosystem dynamics.

#### References

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